Microscopic and qPCR comparison of fungal growth in residential carpets with different carpet fiber materials, at varying relative humidity levels, and presence of household dust

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Abstract

People spend 90% of their time indoors exposed to the microbiome of the built environment. Fungal species are part of this indoor microbiome that have been found to grow on various components of residential homes including house dust, wallpaper, gypsum, insulations, and carpet. Fungal fragments resuspended from carpets are a significant source of human exposure and emissions from metabolic processes can have adverse health effects, such as allergies and exacerbation of asthmatic symptoms. Understanding the process and resulting morphology of fungal growth on residential carpet can provide valuable insights for creating indoor environmental conditions that can improve quality of life for sensitive groups. The goal of this study was to compare fungal growth morphology in residential carpet in varying environmental conditions including relative humidity (RH), carpet fiber material, and the presence/absence of house dust. RH conditions were simulated using three carpet and dust samples extracted from homes in Ohio. Wool, olefin, and nylon carpet fibers were also tested using no dust, sterilized dust, and non-sterile house dust spiked with *Aspergillus versicolor* and *Alternaria alternata* spores obtained from ATCC. Morphology was observed using scanning electron microscopy and confocal microscopy. Fungi were resolved utilizing Uvitex 2B fluorescent stain. qPCR was used to quantify fungal growth in the conditions tested. The presence of house dust was determined to be the most important variable that increased fungal growth. Elevated RH (>90%) and natural carpet fibers compared to synthetic were also factors that increased fungal growth in carpets. The results of this study can provide valuable insights for care providers to look for in patient's homes, motivating improved cleaning practices to remove dust, and guide future building designs to mitigate human exposure to fungi in the built environment. In addition, these results show that synthetic carpet fibers can minimize the growth and proliferation of fungi.

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Introduction

We spend 90% of our time indoors where we are exposed to microbial communities which can have negative impacts on human health [1]. The diversity of this indoor microbiome is influenced by such factors as geographic location, indoor relative humidity, maximum occupancy, presence of pets, and types of material located within the indoor environment [2], [3]. The presence of some fungi in the indoor environment is associated with an increase of asthma severity and allergies through inhalation of fungal spores [4], [5]. In addition to direct inhalation of fungal structures, fungi can also release harmful chemicals, such as microbial volatile organic compounds (MVOCs) into the air [6]. MVOCs, like 1-octen-3-ol which is associated with rhinitis, conjunctivitis, and hay fever, have been found in air samples in residential homes where fungi is present [7]. They may also emit mycotoxins such as aflatoxin as secondary metabolites, which are known carcinogenic compounds [8].

Water-damaged buildings are associated with the presence of certain fungal species, some of which have the ability to grow on many substrates commonly found in residential homes including wood, insulating foam, wallpaper, concrete, and carpet [9]. This is a growing area of concern as major storm events and urbanization are increasing the number of flooding events in residential homes [10]. According to the Carpet and Rug Institute, carpets are the most common flooring material used in built environments accounting for 51% of the total U.S. flooring market [11]. Carpet fibers can act as a sink for microbes that enter through indoor air. Resuspension of microbial particles following abiotic and biotic disturbance from carpets is an important source of human exposure [12]. In addition, fungal growth increases exponentially in carpet containing dust at relative humidity (RH) values of 80% to 100% [13]. Understanding how spore attachment and hyphal growth occurs in varying indoor environmental conditions can provide insights for people afflicted with respiratory diseases. With this knowledge we can better determine how often to vacuum, what carpet materials to

select, and the most ideal indoor RH conditions to maintain. Furthermore, it can provide valuable information for future bio informed building design, which is the promotion of beneficial microbes, as some are critical for the health and well-being of humans, while inhibiting the growth of harmful pathogens [14].

The goal of this study was to gain a better understanding of the morphology of fungal growth on carpets with varying (1) fiber materials, (2) RH levels, and (3) presence/absence of house dust. We hypothesized that the presence of house dust, higher RH conditions, and natural fibers such as wool would stimulate increased fungal growth in carpet in comparison to no dust, lower RH, and synthetic fibers. Residential carpet and dust collected from three homes throughout Ohio (Table S1) were collected and incubated at RH condition of 50, 85, 90, 95, and 100%. Nylon (100%), wool (100%), and olefin (94% polypropylene, 6% nylon) carpets containing no antimicrobial coating were tested to evaluate the effect of fungal growth in different carpet fiber materials. The effects of household dust were also characterized using the three different carpet fiber materials by incubating samples embedded with non-sterile house dust, sterilized house dust, and no dust. Microscopic analysis was used to describe morphology of fungal growth and PCR was utilized for quantification.

Materials and Methods

To study the morphology of fungal growth in residential carpet, three important parameters of the indoor environment were tested during incubation periods of 2 weeks at 25°C. RH was tested at 50%, 85%, 90%, 95%, and 100% from three home carpet samples. Carpet material was tested using three common fibers, wool, nylon, and olefin. Finally, the effect of house dust was studied by inoculating *Aspergillus versicolor* and *Alternaria alternata* onto carpets containing no dust, sterile house dust, and non-sterile house dust.

Carpet Samples

Carpet used for the RH treatment study was collected from three residential homes in Ohio beginning in May of 2016 using a previously described sampling protocol [13]. Household dust used in this study was collected from the residents' vacuum cleaners and filtered through a 300 µm sieve. Carpet samples were stored in airtight plastic bags at room temperature until use in this study. Sieved dust samples were stored in glass beakers covered with parafilm until use. New carpet was also purchased which included 100% nylon, olefin (94% polypropylene, 6% nylon), and 100% wool carpet fibers which contained no antimicrobial coatings for use in this study. All carpet samples were cut into 5cm x 5cm squares and sterilized by autoclaving at 121°C for 1 h and baking at 100°C overnight (~12 h) prior to incubation. In carpet samples containing the household dust, a modified ASTM method F608-13 and 12 cm long, 1440 g steel pipe was used to embed 50 mg of household dust into each carpet square avoiding a 1 cm area bordering the edge of the sample. [13] Dust used RH samples were collected from the indoor environment from each corresponding site. In the materials and dust test, site 1 dust was used to study its effects on fungal growth. A total of three samples were used for each fiber material: one with no dust, one with dust sterilized using the method described above, and one with pure site 1 house dust. An outline of sample conditions tested are summarized in Figure 1.

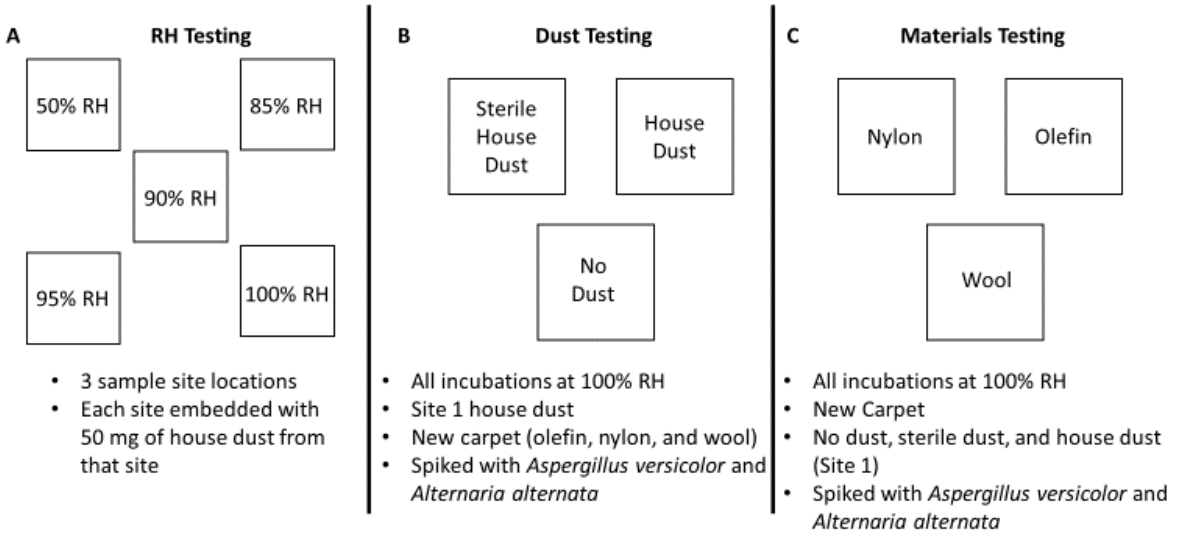

Figure 1: Summary of samples and conditions: Each square represents a 5 cm x 5 cm carpet coupon.

Fungal Strains

Freeze-dried *A. versicolor* and *A. alternata* strains were purchased from ATCC, item number 9577 and 66981 respectively, and rehydrated in sterilized distilled H_2O overnight (~12 h). The rehydrated fungal strains were vortexed for 15 seconds and 10 µL aliquots were placed onto Potato Dextrose Agar (PDA) [Difco Potato Dextrose 24 g; Agar 15 g; Distilled H_2O 1 L] culture plates. The PDA plates were allowed to incubate for 2 weeks at 25°C. Heavy sporulation occurred with *A. alternata*; in contrast, minimal spore formation occurred with *A. versicolor* on PDA. To promote heavy sporulation in *A. versicolor*, spores were aseptically transferred to a Lignocellulose Agar (LCA) [Glucose 1 g; KH_2PO_4 1 g; $MgSO_4 \bullet 7H_2O$ 0.2 g; KCl 0.2 g; $NaNO_3$ 2 g; Yeast Extract 0.2 g; Agar 13 g: Distilled H_2O 1 L] [15]. Media was supplemented with 0.025 g of chloramphenicol (Sigma Aldrich) to prevent bacterial contamination. The LCA plates were allowed to incubate an additional 2 weeks at 25°C. *A.s versicolor* spores were harvested from the LCA plates by carefully tapping spores with a FLOQSwab (Copan) saturated with a

Phosphate-buffered saline (PBS, Sigma Aldrich) and Tween-20 (Fisher Bioreagents) solution [PBS 1600 µL; Tween-20 1.6 µL] to obtain a final concentration of 10^6 spores/µL. The previous method was insufficient to remove *A. alternata* as their spores were more tightly bound to hyphal structures. Instead a modified spore charge method was used in which a PBST solution [10 mL PBS, 10 µL] was poured into each PDA plate, scraped with an inoculating loop, and the spore charge was then poured into a flask containing 2 mm garnet beads (ASTM G26). This solution was shaken vigorously to release spores from the hyphae and then filtered through sterile wool. This process was repeated to obtain a 10^6 spores/µL solution. Spores were resolved and counted by a stained solution [Crystal Violet (Sigma) 10 µL; Tween-20 (Fisher Bioreagents) 10 µL; Spore Solution 10 µL; Distilled H_2O 970.µL], 10 µL of which was aliquoted onto a 3 separate InCyto DHC-N01-5 Neubauer Improved C-Chips and viewed with a Labomed epifluorescent microscope with a 20x air objective lens.

Relative Humidity Control

Salt solutions were used to control RH conditions inside of the incubation chambers and were comprised of $MgCl_2$ and NaCl. For 50% RH, 44.84 grams of $MgCl_2$ was added to 100 mL of DI water. For 85, 90, and 95% RH, a total of 46.76, 35.89, and 27.54 grams of NaCl was added to 100 mL of DI, respectively. The water activity of each salt solution was measured on a Aqualab 4TE Dew Point Water Activity Meter (Decagon Devices, Pullman, WA, USA). The water activity measured in each salt solutions would represent the RH equilibrium in each incubation chamber. 100% RH was achieved by using deionized water (DI) only. Onset HOBO loggers (Bourne, MA USA) were placed in the incubation chambers to confirm RH conditions stayed constant during the incubation period.

Inoculation

Based on the experimental design shown in Figure 1, some samples were inoculated with known species and others were not. No inoculation of fungi was performed on

samples for RH testing. All fungal growth occurred from spores already present in each sites dust which was embedded into the carpet. Pure *A. versicolor* and *A. alternata* spores were inoculated onto the samples containing no dust, sterilized dust, and non-sterilized dust in each of the three carpet materials tests (wool, nylon, and olefin). Inoculation of spores utilized a Medline Aeromist Compact Nebulizer compression kit. Spore solutions of *A. versicolor* and *A. alternata* were diluted in PBS to a 10^6 spores/mL concentration. 3 mL of the diluted spore solution was placed into the nebulizer tank. The 5 cm x 5 cm carpet squares were placed, fiber side up, into a 1 L glass jar. A 5/16" hole was drilled into the glass jar's aluminum lid. A flexible plastic tubing was attached to the nebulizer tank and feed through the newly drilled hole in the aluminum lid. The compressor was turned on for 10 minutes at a flow rate of 0.18 mL/min to release the spores into the 1 L chamber. The chamber was then allowed to settle for an additional 10 minutes before placing carpet samples into their incubation chambers. The inoculation setup is shown in Figure 2.

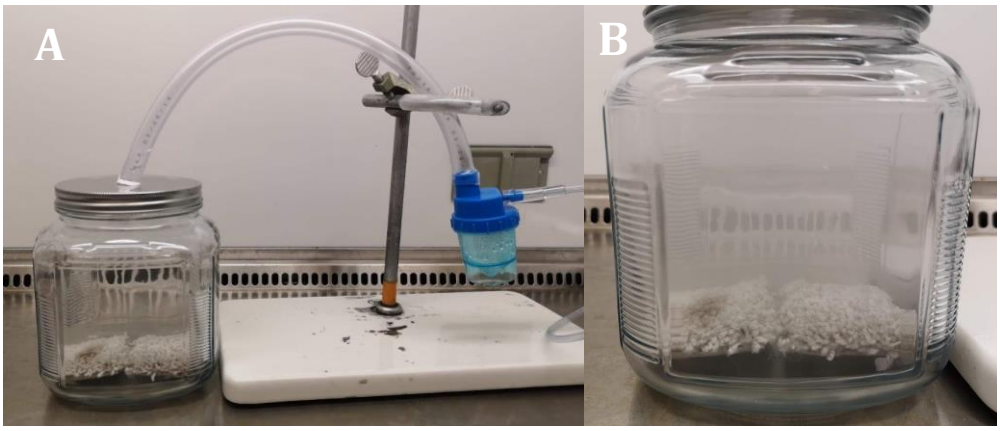

Figure 2: Inoculation setup. (A) time = 0 and (B) time = 10 minutes.

Incubation

All samples were incubated in sterilized 2 L glass jars at 25℃ for 2 weeks in a VWR incubator as shown in Figure 3. Each carpet sample from each site was placed into the chamber and separated by tinfoil that was baked at 550℃ to prevent any cross-contamination of the samples. 100 mL of the salt solutions were placed inside of each

chamber to simulate each RH condition being tested. The top of each glass jar was covered with parafilm which was checked daily to keep moisture in and prevent CO_2 accumulation.

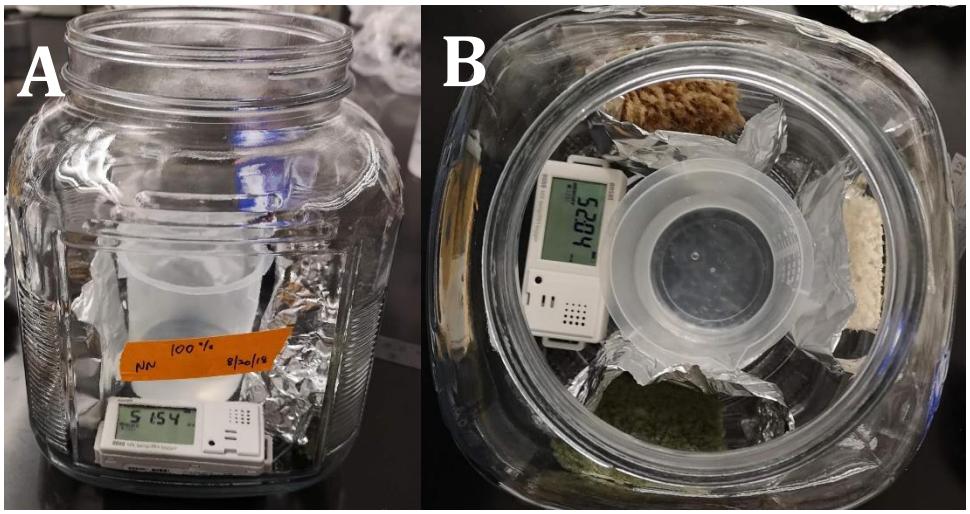

Figure 3: Incubation Chamber with samples, HOBO logger, and salt solution from (A) side view and (B) top view

Microscopy Preparation

After 2 weeks of incubation, carpet fibers were cut from the sample squares using aseptic techniques and approximately 1.25 mg placed on Fisher Scientific glass microscope slides (25 x 75 x 1 mm). For confocal and light microscopy, fixation was achieved using a 4% Paraformaldehyde (PFA) Solution in PBS (Affymetrix). PFA (100 µL) was aliquot on to the sample slide and allowed to sit for 2 h. Uvitex2B stain (50 µL) was applied directly to the fixed samples and allowed to sit for 5 min. PBS was gently applied to the samples to rinse and was carefully pipetted off to prevent removing fungal structures from being removed. All samples were stored in the dark until microscopic analysis was performed on that same day. Uvitex 2B is a non-selective stain that has been shown to be an effective for highlighting fungal structures by binding to chitin [16]. Uvitex 2B is a fluorescent stain that excites under DAPI filtration (~385 nm) and emits a blue wavelength (~480 nm). Sample preparation for Scanning Electron Microscopy (SEM) imaging consisted of extracting fibers, placing them on an aluminum stud with

double-sided black carbon tape. The samples were then sputtered with 10 nm of gold to dissipate heat from the focused electron beam.

Microscopy and Image Analysis

Fluorescent microscopy analysis was performed on a Nikon AR1 Inverted Confocal at the Campus Microscopy and Imaging Facility. SEM imaging was performed on a Apreo LoVac Scanning Electron Microscope at the Center for Electron Microscopy and Analysis. Both facilities were located on The Ohio State University's Main Campus in Columbus, OH.

Quantification by qPCR

Overall fungal quantity on each sample was measured using quantitative polymerase chain reaction (qPCR) on an Applied Biosystems Quantstudio 6 Flex (Fisher Scientific, Waltham, MA, USA) and analyzed using Quantstudio Real-Time PCR Software v1.2. 10 µM Fungal forward-primer FF1 (5´-GTTAAAAAGCTCGTAGTTGAAC-3´) and 10 µM reverse primer FR1 (5´-CTCTCAAT-CTGCAATCCTTATT-3´) were used as a "universal" fungal primer derived from the 18 S rRNA gene homologous to fungi, but not other organisms [17]. For each sample, a 25 µL reaction buffer was used which included 2 µl of DNA (10X dilution) from each sample and 23 µL Applied biosystems SYBR® Green PCR Master Mix. qPCR conditions included 1 cycle of 50°C for 2 minutes and 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Before running samples on qPCR, extraction efficiencies for each fiber material was determined by spiking 20 µL of a 10^6 spores/µL solution of *A. versicolor* onto 50 mg of each fiber material. The spiked fibers and 20 µL of the spore solution were put through the Qiagen DNA extraction process and run on qPCR. DNA was extracted from each carpet square that included 50 mg of fibers, biomass, and dust. Each DNA extract was then run in triplicate on the qPCR. Standards for the qPCR runs were made from an *A. fumigatus* spore solution ($2.288*10^6$ spores/µL) using 6 points in duplicate with a 10X serial dilution.

Statistical Analysis

All calculations for qPCR data was done in Microsoft Excel. Statistical analysis comprised of sample population means and propagation of errors to obtain one standard deviation for all samples analyzed. P-values to determine statistical differences in carpet fiber materials were calculate with JMP software using a Tukey-Kramer test.

Results

Microscopic evaluation showed fungi in carpet fibers, including fungal spores and hyphae. Spore chains, septate hyphae, and philiades were also observed indicating asexual reproduction of fungal species within the carpet materials (Figure 4). qPCR analysis additionally quantified spore equivalents per mg of fiber-dust in most samples.

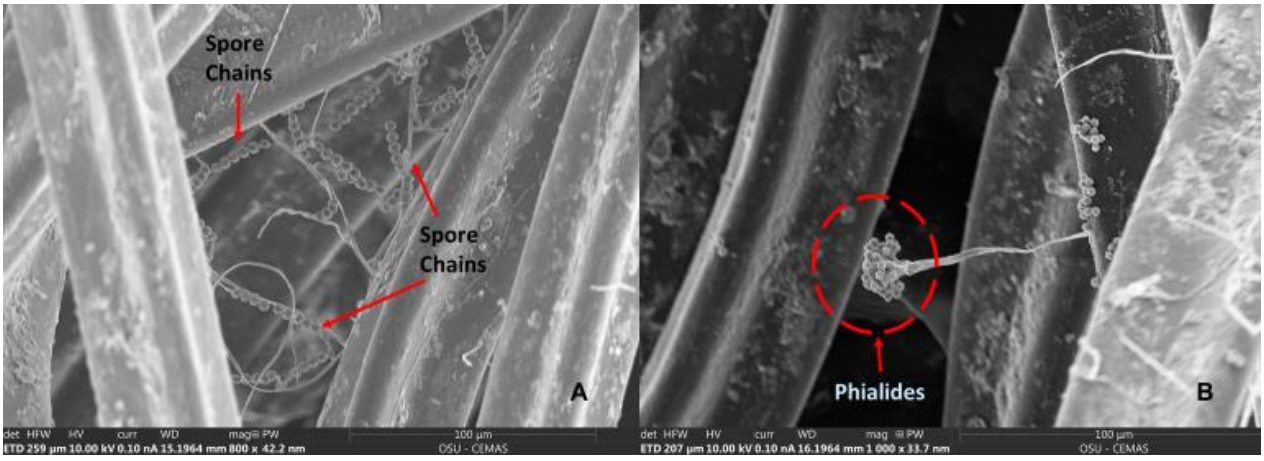

Figure 4: SEM images incubated at 95% RH for 2 weeks at 25°C showing (A) spore chains and (B) Phialides which are signs of fungal asexual reproduction

Relative Humidity

In each microscopy observation, 50% RH showed very few fungal spores and no growth for all sample sites. Fungal spore quantity slightly increased at 85% RH, while around 90% RH hyphae were observed indicating growth. From 95% to 100% RH, fungal

growth had covered most of the carpet fibers. At 95 and 100% RH similar quantity were observed, however, presence of phialides and spore chains was much greater at 95% RH. No growth was observed in site 3 samples at 90, 95, or 100% RH conditions. As observed in the confocal microscopy analysis, fungal hyphal networks directly on the fiber increase in size and numbers as RH increases (Figure 5).

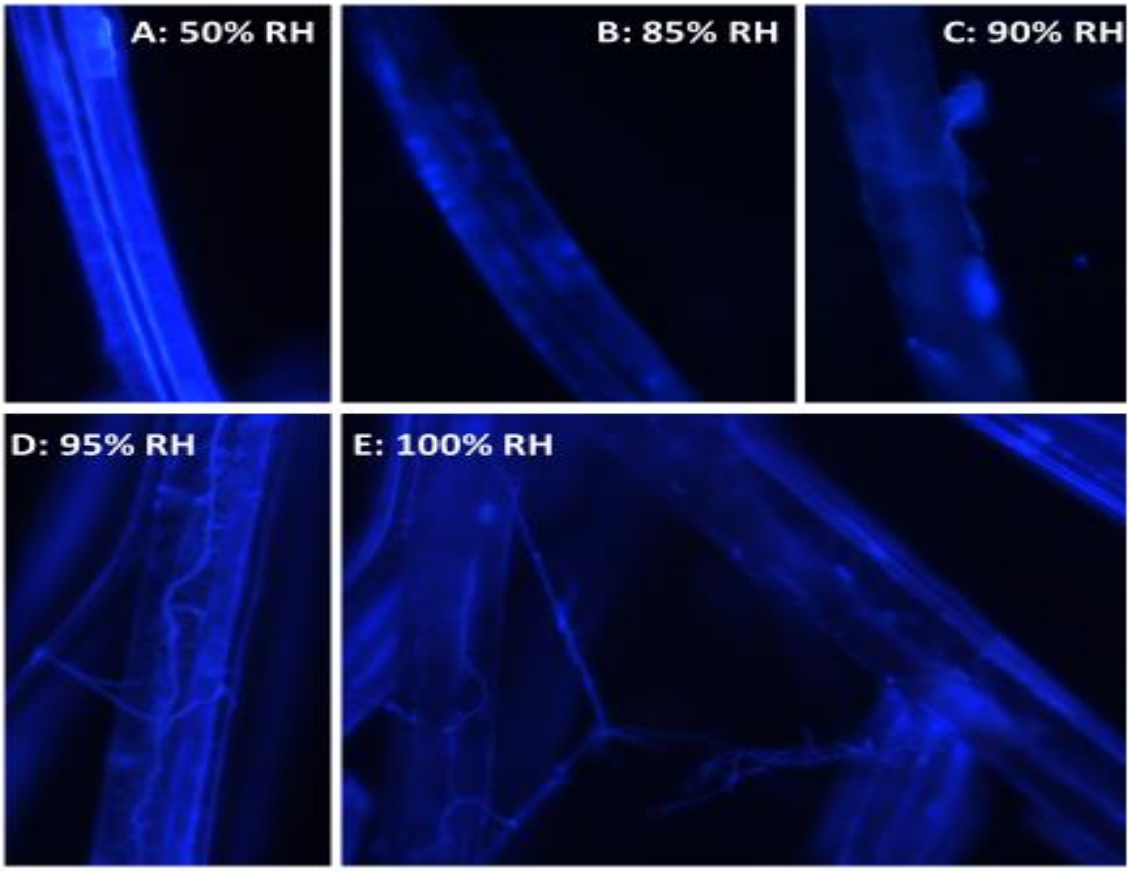

Figure 5: Confocal images of fungal growth on nylon carpet fibers. Samples were fixed with 4% PFA, stained with Uvitex 2B, and gently washed with PBS. Samples were incubated at 25°C for 2 weeks at (A) 50%, (B) 85%, (C) 90%, (D) 95%, and (C) 100% RH conditions.

SEM imaging showed a similar trend, with little to no growth at 50% and 85%, beginnings of hyphal structures at 90% RH, and full growth at 95% and 100% RH. The majority of fungal spores may be *A. sydowii* due to their globose to sub-globose spore morphology characterized by a spiny surface ornamentation. This species was also identified to be the most abundant species in Illumina ITS sequencing of site 1 house

dust (Table S2) [18]. Figure 6 shows an example of samples at low (50%-90%) to high (95-100%) RH conditions.

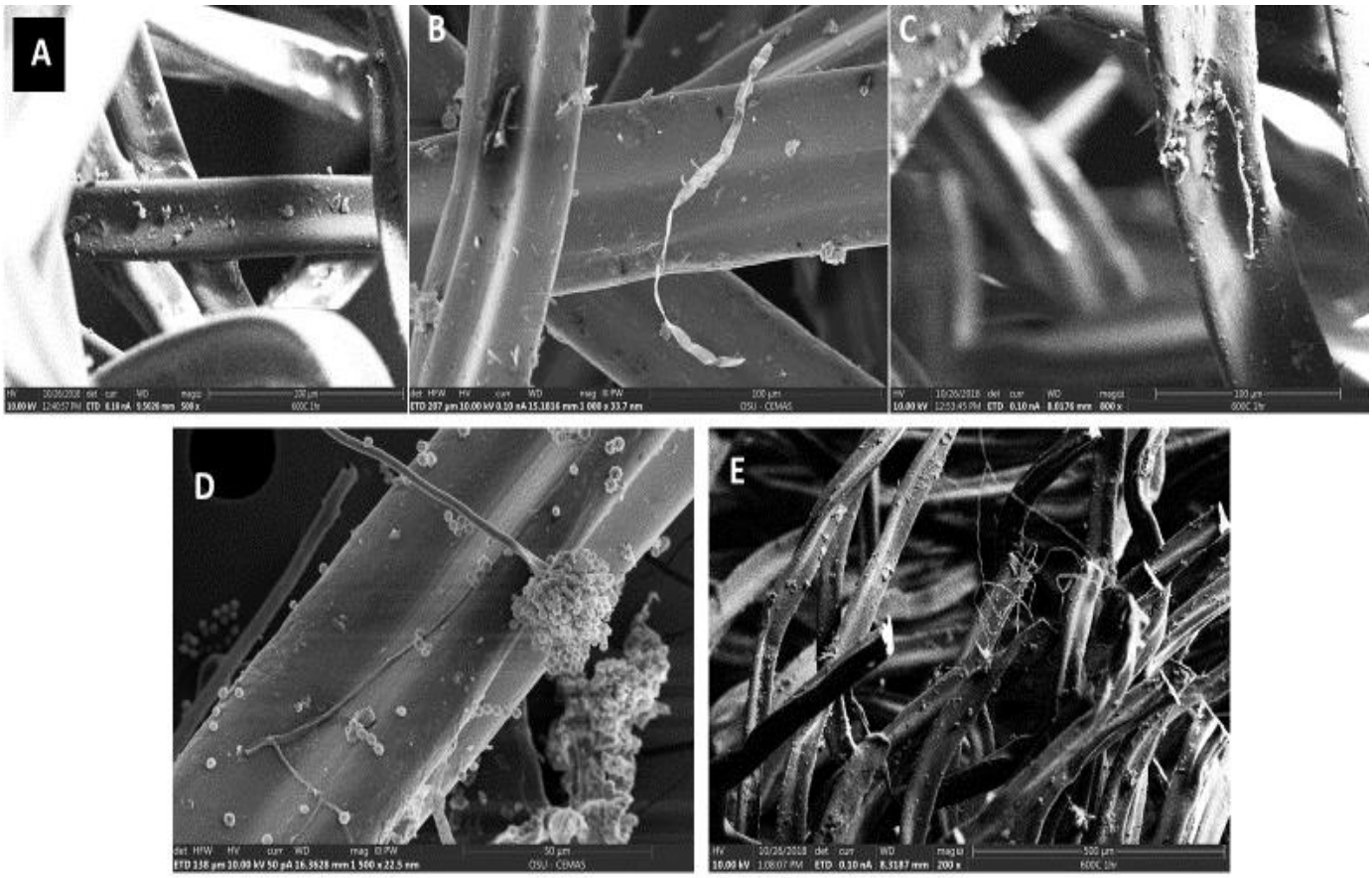

Figure 6: SEM images of (A) dust on fibers, no growth at 50% RH, (B) small hyphae on fiber at 85% RH (C) spores on fiber with small hyphal structures at 90% RH, (D) spores, large hyphae, and phialides at 95% RH, and (E) large hyphal networks and spores on fibers at 100% RH.

Carpet Fiber Materials

Wool, nylon, and olefin carpet fibers were spiked with sterilized dust and inoculated with *A. versicolor* and *A. alternata* spores. Our strain of *A. versicolor* showed no signs of growth on any of the fiber materials despite previous studies showing growth in house dust of this species [6]. *A. alternata* was able to grow in all carpet fiber materials tested. Growth appeared to most abundant in olefin fibers showing large spore quantities and large hyphal structure networks. Wool exhibited the second highest growth with

moderate to large hyphal structures and spore chains. Nylon showed the least amount of growth with minimal spore attachment and small hyphae. Figure 7 shows *A. alternata* growth on each carpet fiber material via SEM imaging.

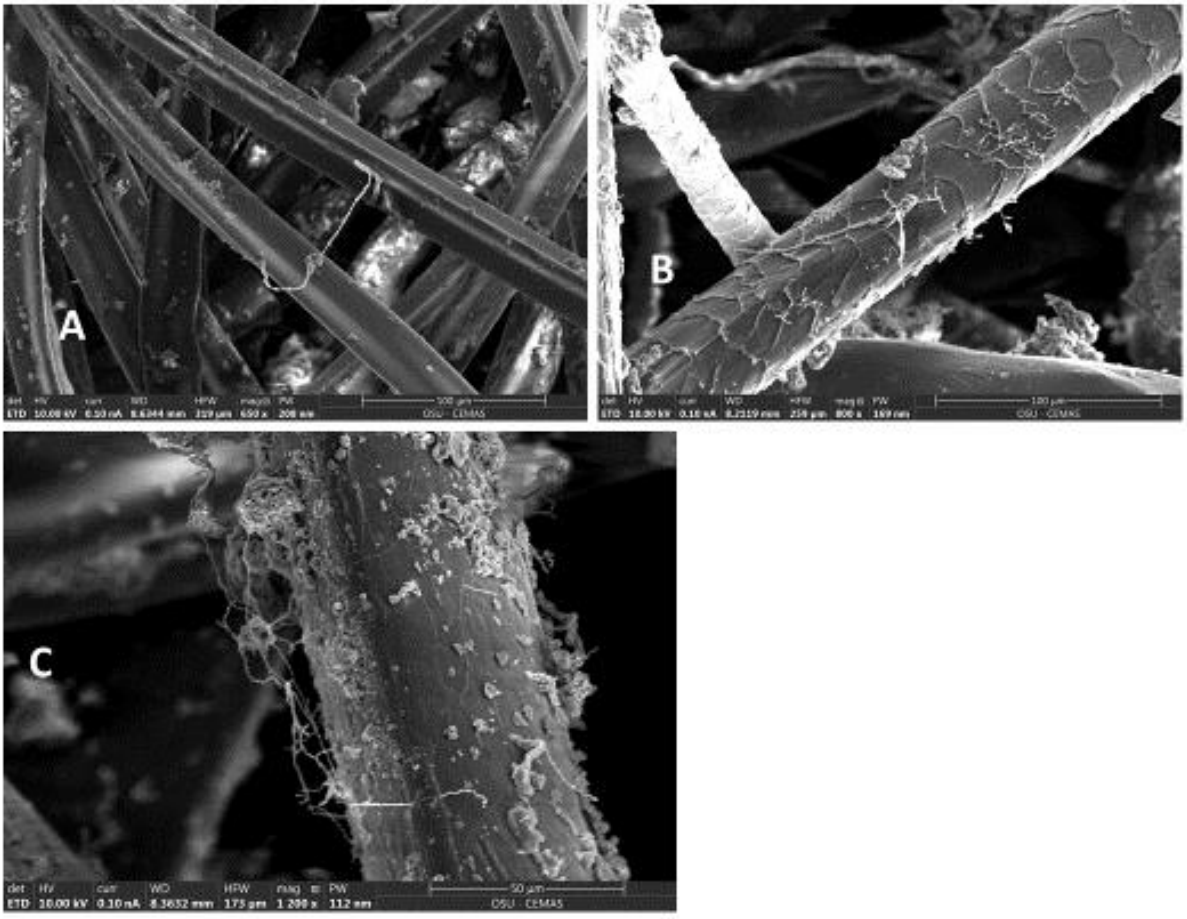

Figure 7: SEM images of *A. alternata* on (A) nylon, (B) wool, and (C) olefin carpet fibers. All samples were incubated at 25°C for 2 weeks at 100% RH. Fibers were cut carefully from carpet and coated with 10 nm of gold SEM imaging under high vacuum.

House Dust and Carpet Fibers

The effect of house dust presence on fungal growth was determined by inoculating *A. versicolor* and *A. alternata* onto carpet samples and incubating at 100% RH with one containing no dust, one containing sterilized house dust, and one containing non-sterile house dust. As with the materials testing, *A. versicolor* showed no growth in all samples,

and *A. alternata* showed no growth on samples containing no dust. However, considerable growth and spore content was observed on carpet fibers containing both the sterile and non-sterile house dust with no discernable difference in morphology in *A. alternata*. Through SEM and confocal microscopy analysis, qualitative observations showed that the quantity of *A. alternata* was appreciably lower and other fungal species were also observed in samples with non-sterile dust. Some examples of fungal interaction with house dust are shown in Figure 8.

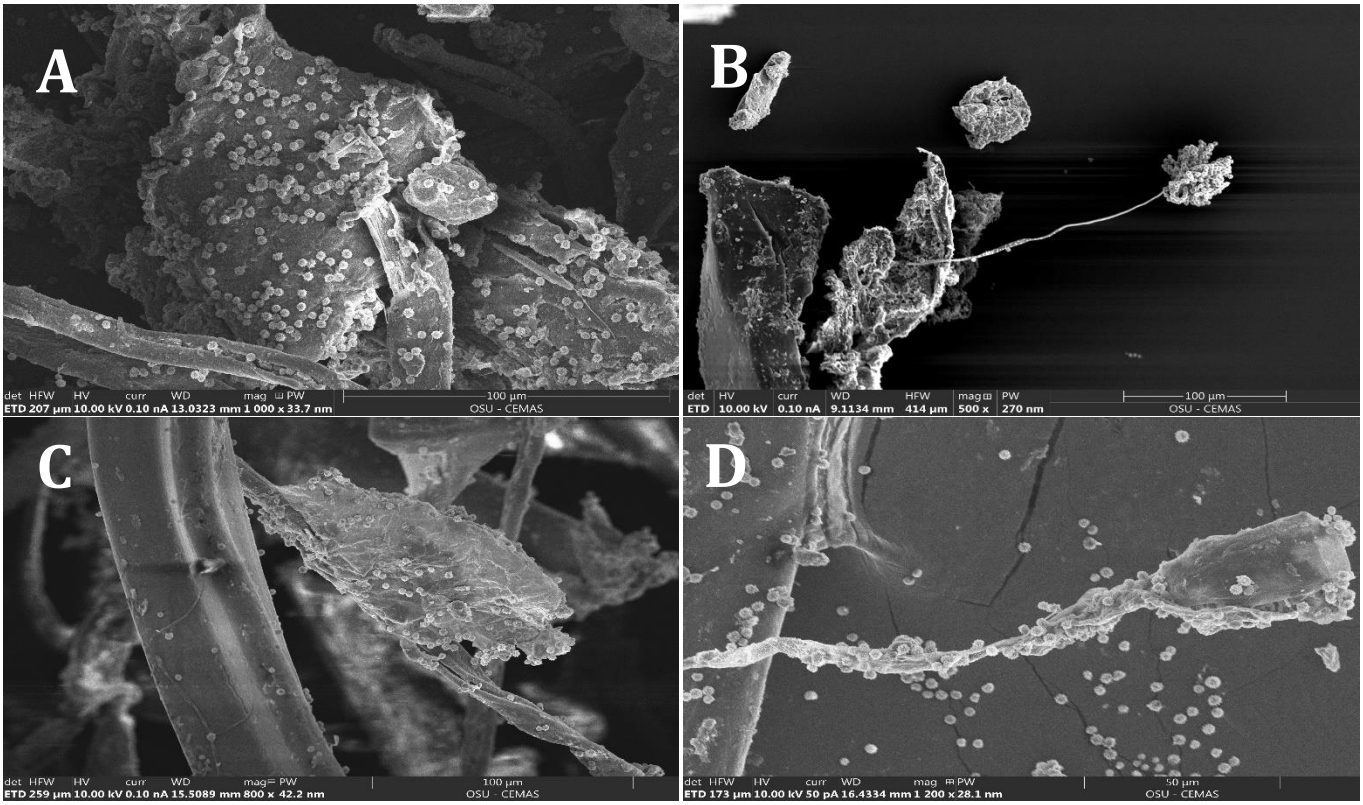

Figure 8: House Dust and fungal growth at 95% RH. (A) fungal spores on dust particle, (B) hyphae with phialides growing out from house dust at 100% RH, (C) fungal spores on dust particle at 100% RH, and (D) fungal spore attached to possible hair fiber or dust strand at 95% RH.

qPCR Analysis

DNA extraction efficiencies for nylon, olefin, and wool fiber were calculated using Equation 1 and deviations were determined by using the propagation of errors (Equation 2). Nylon and wool fiber materials showed ~100% extraction efficiency of

spike DNA compared to the amount recovered from the spike with no carpet fibers. The DNA extraction method was not as efficient with Olefin fibers coming in at 56% efficiency (Table 1).

$$Efficiency\ (\%) = \frac{Quantity\ from\ fiber\ material}{Quantity\ from\ spore\ solution} * 100 \quad \textbf{(1)}$$

$$\sigma = \sqrt{(\sigma_{spore\ solution})^2 + (\sigma_{fiber})^2} \quad \textbf{(2)}$$

Table 1: qPCR Carpet Fiber DNA Extraction Efficiency*

Sample	Quantity Mean (spore eq/mg fiber-dust)	Quantity SD	Efficiency (%)	SD (%)
A. fumigatus	**109631**	**18118**		
50 mg Wool	**112224**	**7998**	**102**	**18**
50 mg Olefin	**61839**	**1588**	**56**	**9**
50 mg Nylon	**109678**	**4345**	**100**	**17**

qPCR values for each sample were calculated to units of spore equivalents per mg of fiber-dust using Equation 3.

$$\frac{Spore\ Equivalents}{mg\ of\ Fiber{-}Dust} = \frac{Spore\ Equivalents}{\mu L\ of\ DNA\ Extract} x \frac{50\ \mu L\ DNA\ Extract}{50\ mg\ of\ Fiber{-}Dust} \quad \textbf{(3)}$$

In this analysis, fiber-dust refers to the combination of carpet fiber material, embedded house dust, and biomass grown during incubation which was extracted from the original 5cm x 5cm carpet sample. Carpet materials were analyzed by qPCR by using values obtained from each fiber material embedded with sterile house dust and inoculated with *A. alternata.* As Figure 9 shows, Wool fibers showed the most fungal growth, followed by nylon and then olefin fibers. Wool is statistically different from nylon ($p = 0.0014$) and olefin ($p = 0.0006$), while no statistically significant difference between nylon and olefin were shown ($p = 0.5578$).

* Spore equivalents refers to the DNA extracted from each sample that can include spores, hyphae, and other fungal structures. Fiber-Dust is the combination of carpet fiber material, dust particles, and biomass growth in which the DNA was extracted from.

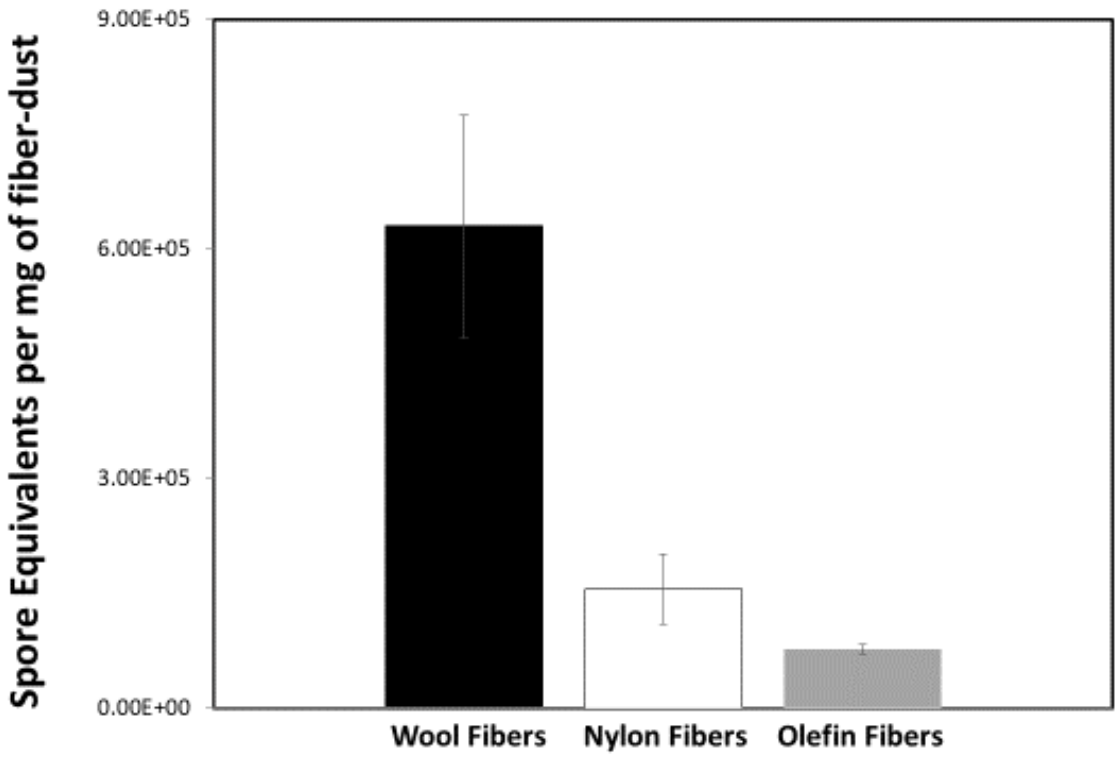

Figure 9: qPCR results for fungal growth with respect to carpet fiber materials. Error bars displayed are standard deviation of triplicate samples.

For each carpet fiber material, the effects of no dust, sterile dust, and non-sterile dust on fungal growth were quantified. In each case, non-sterile dust was observed to promote the most fungal growth. In addition, sterile dust provided more growth than no dust but much less than non-sterile dust for each carpet fiber materials (Figure 10).

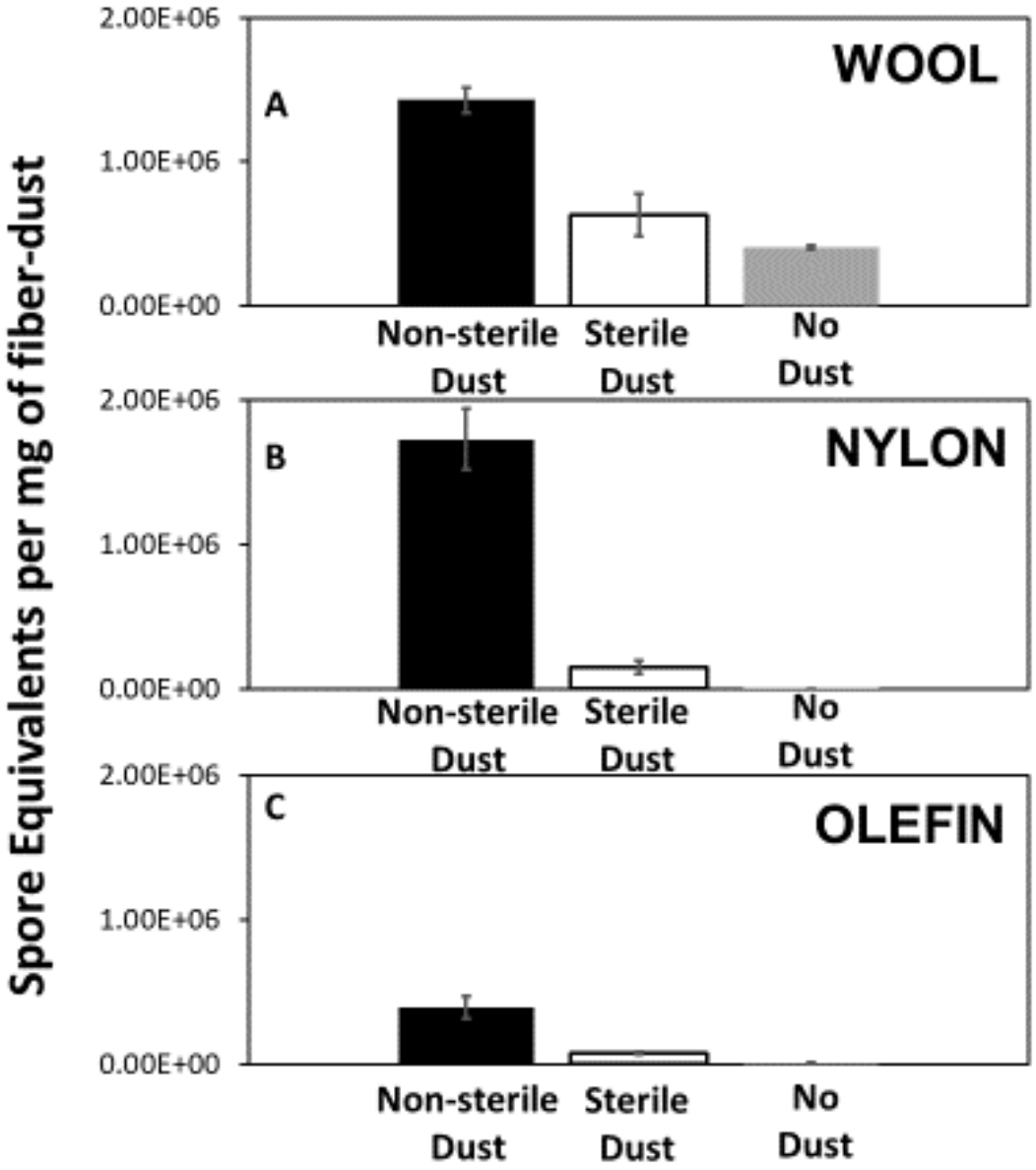

Figure 10: qPCR results for the effects of dust on fungal growth on (A) wool, (B) nylon, and (C) olefin carpet fibers. All samples incubated for 2 weeks at 25°C and 100% RH. Site 1 dust was used for sterile and non-sterile dust inoculations.

qPCR results showed fungal growth at low values from 50% to 90% RH in site 1. This is followed by an exponential increase in growth at 95% and 100% RH. Site 2 showed low fungal growth for 50%, 85%, 90%, and 100% RH conditions. However, at 95% RH a 10^6 spore equivalents/mg fiber-dust increase was observed (Figure 11). Site 3 showed no

growth at any RH condition. Sites 2 and 3 will be run on qPCR again to test for inhibition given the results presented.

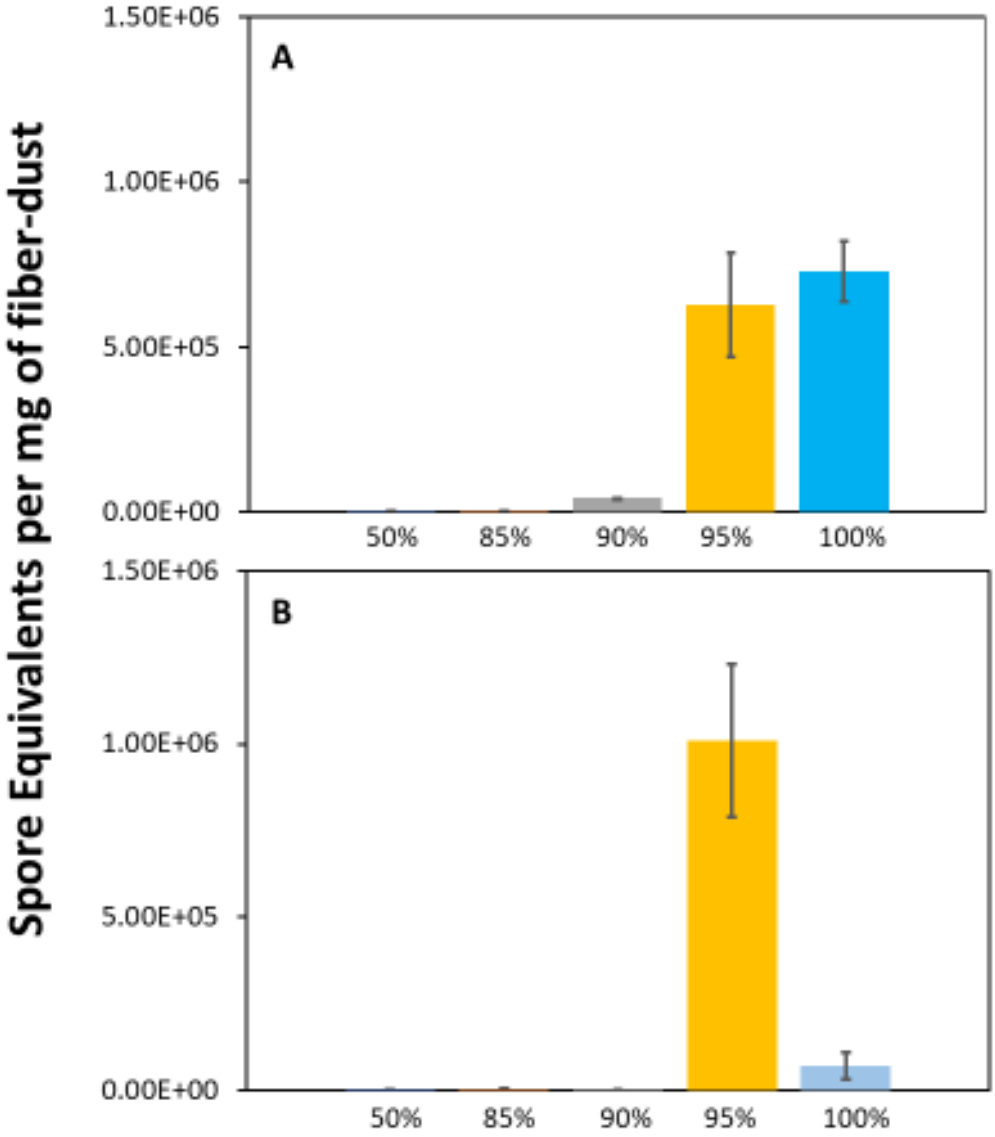

Figure 11: qPCR results for (A) Site 1 and (B) Site 2. Each site was incubated at 25°C for two weeks and embedded with house dust from their respective sites.

Discussion

RH conditions, carpet fiber material, and presence of house dust all have significant impact on fungal growth in carpet. Elevated RH conditions of greater than 90%, the presence of house dust, and natural carpet fibers all favored increased fungal growth,

that included spore content, as well as other indicators of asexual reproduction such as septa in hyphae, spore chains, and phialides. This aligns with previous qPCR results of fungal growth in house dust [13]. The presence of these fungal structures shows that fungi can proliferate once established in these conditions, regardless of the source of the initial deposition of these fungal species into the indoor environment.

The presence of house dust was determined to be the most important factor contributing to fungal growth in carpet. qPCR analysis showed no fungal growth on carpet samples containing no house dust that were spiked with *A. versicolor* and *A. alternata*, with the exception of *A. alternata* capable of growing on wool fibers. In addition, microscopy and qPCR analyses showed abundant growth on carpet samples containing sterilized and non-sterilized house dust, regardless of carpet fiber material compared to samples with no dust. House dust can be an important source of nutrients such as organic carbon, nitrate, phosphate, and sulfate providing levels 4 times greater than the stochiometric requirements for microbial growth [13]. House dust is also highly variable in size and chemical contents based on geography, occupancy, presence of pets, and seasons which can all effect the quantity and diversity of microbial communities [7]. Fungal species, such as *A. versicolor* and *A. fumigatus,* can grow on many inorganic materials, especially in hygroscopic conditions and in the presence of absorbed dust that serves as a suitable substrate [19], [20]. This highlights a need for better understanding of house dust chemistry and a general model which links it to fungal growth at the various environmental conditions in this study.

Wool is a fibrous protein, called keratin, comprised of amino acids. Several strains of fungi isolated from soils, including *Trichophyton* sp., *Fusarium* sp., *Trichoderma* sp., and *Cladosporium* sp., have been observed to have the ability to metabolize wool fiber substrates utilizing kertinase enzymes to cleave di-sulfur bonds [21]. Cladosporium species were observed in Site 1 dust, which was used on the wool carpet in this study (Table S2), although in low abundance (Figure 8). It is also possible that other species may have keratin degrading ability, such as *A. alternata* as demonstrated by its ability to grow on wool carpet fibers containing sterilized dust substrate and no dust (Table 9a).

These data suggest potential signs of keratin degradation by *A. alternata* were observed in wool fibers with sterilized dust (Figure 12), but this would need to be validated in future studies. White rot fungus, utilizing manganese peroxidase as a catalyst, has the ability to degrade nylon and with gamma irradiation assistance, the potential to degrade polypropylene [22], [23]. It is unknown how much fungal growth typically contributes to wear on carpets, but this could be a subject of future study. This information can provide guidance for consumers, especially those sensitive to allergies or with asthma, for purchasing carpet materials that may reduce their risk for harmful exposure to fungi and their metabolites.

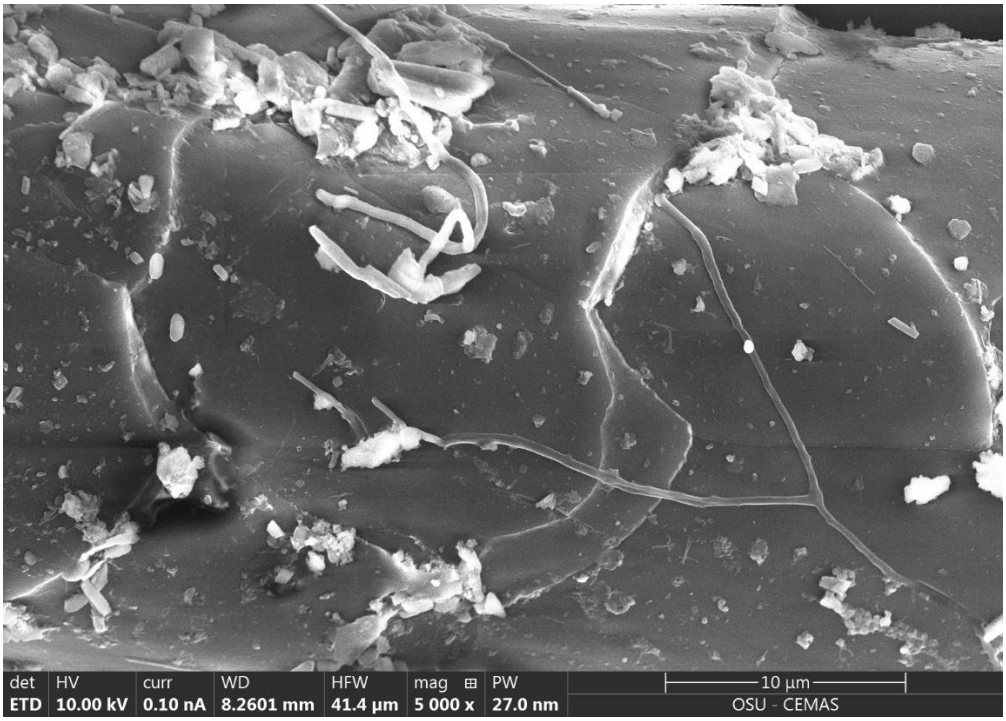

Figure 12: *A. alternaria* penetrating wool carpet fiber. Carpet embedded with sterilized dust and inoculated with *A. alternaria*. Incubated for 2 weeks at 25°C and 100% RH.

The presence of phialides in carpet samples with dust and at elevated RH (90-100%), poses significant risk of direct inhalation exposure to spores that can have negative impacts on human health. The spores that were attached to carpet fibers did not appear to have any physical attachment method, but more likely were held in place by electrostatic charge (Figure 13) [24]. These spores are vulnerable to release in the air

by disturbances such as walking across the carpet or breezes from fans/ventilation systems. In order to mitigate fungal growth to prevent adverse health effects, occupants may prevent fungal growth by keeping the RH less than 50%, cleaning carpets to reduce dust burden more often, taking care to detect water leaks that can provide excess moisture and so on. Several of the RH levels used in this study are higher than what may be experienced in a typical home. However, these levels are not unreasonable in a bathroom, next to a water leak, or other suboptimal conditions.

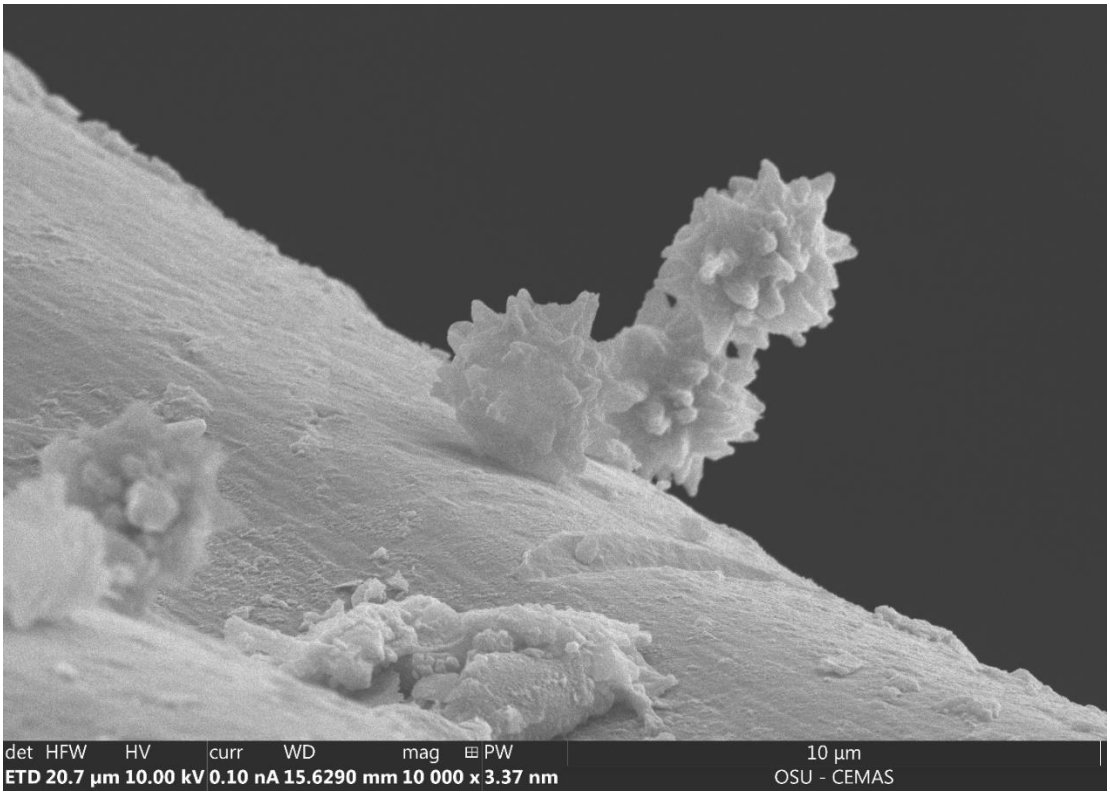

Figure 13: Putative *Aspergillus sydowii* spores resting on nylon carpet fiber. Carpet embedded with house dust from Site 2. Incubated for 2 weeks at 25°C and 95% RH.

Changes to existing buildings may be difficult to correct, but future building can readily implement these findings into their designs. By selecting carpet fibers that restrict fungal growth, installing ventilation ducts away from the carpet to prevent resuspension, and designing to keep unnecessary moisture outside future building designs can create a non-ideal environment for fungal growth and resuspension. Microbiologists and

architects have already realized the potential of utilizing the microbiology of the built environment to address real world health and sustainability issues [25].

Aspergillus versicolor

A. versicolor has been observed to grow in carpet dust in damp indoor environments [26]. However, no growth of *A. versicolor* was observed on carpet samples inoculated in this study. Further investigation into the *A. versicolor* strain used in this study (ATCC 9577) was isolated from a human lesion in New York City, USA in 1935 [27]. This suggests that *A. versicolor* species previously found in carpet may have evolved to survive in these materials in a way that the isolate used in our study did not. In the future, we can try to inoculate the carpet samples with a strain isolated from the indoor environment.

Limitations

Carpet fibers analyzed via microscopy may not be a total representation of fungal morphology in the whole carpet sample for each condition tested. The fibers (1.25 mg) that were used in microscopy analyses were relatively small compared to the total number of fibers on the 5cm x 5 cm carpet coupons. Furthermore, qPCR, confocal, and SEM imaging were all performed on one sample for each condition which means fungi may have been removed during confocal analysis and may have not identified seen via qPCR or SEM. Additionally, the use of stains for fungal quantification and identification yields highly variable results. This is due to the absorbent nature of the carpet fiber materials which retained the stain creating autofluorescence in many samples. As shown in Figure 10, each site displayed very different fungal growth patterns with RH changes. This may be attributed to the chemical composition of each site's house dust, which was not known for this study. In addition, qPCR values are reported in spore equivalents and will not account for differences in amplification bias or gene copy number between species [28],[29].

Conclusions

Fungal growth on carpets in residential homes can increase severity of respiratory diseases, such as asthma, and decrease quality of life for those afflicted with such diseases. Understanding how fungal growth occurs on carpet fibers can be beneficial to learn how to inhibit the proliferation of harmful species and create more effective preventative measures.

This study provides a novel approach to observing fungal morphology in residential carpet fibers. Future work can expand on this study by developing sample fixation methods that cause less disturbance to the natural growth of carpet fibers. New fungal stains that do not react with carpet fiber materials would also be beneficial for future studies of morphology and growth on fibers.

Supplemental Information

Table S1: RH Sample Site Data

Site ID	1	2	3
Weather	Rainy/Cloudy	Sunny, Warm	Sunny/Cold
Outdoor Temperature	75°F	18°F	7°F
Outdoor Relative Humidity	85%	37%	80%
Indoor Temperature	75°F	15°F	19°F
Indoor Relative Humidity	50%	42%	53%
Water Damage or Mold Growth	No	No	No
Type of Room Sampled	Living Room	Living Room	Bedroom
Room Observations	Lower level of home, fireplace	Fireplace, large windows	Small, windows
Number of Occupants	5	5	4
Adults	5	3	4
Children	0	2	0
Number/Type of Pet	1 Cat	2 Dogs, 2 Cats	2 dogs, 1 cat
Smoking in house?	No	No	No
How many cigarettes per day?			
Total Home Area	N/A	2800	3500
Total Area of Sampled Room	N/A	200	144
Heating System	Furnace	Boiler	Gas Forced Air
Open Windows in:			
Spring	Yes	Yes	Yes
Summer	Yes	Yes	Yes
Fall	Yes	Yes	Yes
Winter	No	No	No
Air Conditioner in Summer Months	Yes	No	Yes
What month do you turn it on?	June	N/A	May
What is the last month you use it?	October	N/A	Sept
What type? (Window unit or full house)	Both	N/A	Central AC
How often do you vacuum carpet?	Every Other Week	2 Weeks	1 week
Approximate age of carpet?	11+ Years	20 years	14 years

Table S2: Illumina ITS Sequencing of House Dust Site 1 and Site 2

SITE 1		Site 2	
Fungal Species	**QTY**	**Fungal Species**	**QTY**
Aspergillus sydowii	**38096**	***Penicillium chrysogenum***	**415162**
Penicillium chrysogenum	**10631**	***Cladosporium sphaerospermum***	**293452**
Epicoccum nigrum	**6357**	***Acremonium charticola***	**180114**
Aspergillus pseudodeflectus	**6243**	***Aspergillus unguis***	**114767**
Cladosporium sphaerospermum	**4609**	***Cladosporium halotolerans***	**81889**
Alternaria alternata	**4303**	***Sterigmatomyces halophilus***	**79234**
Cladosporium delicatulum	**3240**	***Gibberella intricans***	**73516**
Acremonium alternatum	**2160**	***Aspergillus austroafricanus***	**39821**
Aspergillus melleus	**1748**	***Verticillium dahliae***	**35329**
Chalastospora ellipsoidea	**1459**	***Aspergillus sydowii***	**30836**
Alternaria infectoria	**1438**	***Epicoccum nigrum***	**24914**
Gymnascella confluens	**1280**	***Aspergillus pseudodeflectus***	**23893**
Penicillium citrinum	**766**	***Nothophoma anigozanthi***	**12661**
Alternaria chlamydospora	**733**	***Cladosporium delicatulum***	**12661**
Mycosphaerella tassiana	**579**	***Toxicocladosporium irritans***	**11232**

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